in the presence of a test compound. The cells can then be observed to determine if the WNV or other viruses including *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, induces apoptosis. A control may be provided in which cells are contacted with WNV or other viruses including *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, in the absence of test compound. A further control may be provided in which the test compound is contacted with cells in the absence of WNV or other viruses including *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof. If the cells contacted with WNV or other viruses including *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, in the presence of the test compound do not undergo apoptosis, then an anti-apoptotic activity is indicated for the test compound. This can be confirmed if cells contacted with WNV or other viruses including *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, in the absence of the test compound detectably undergo apoptosis and the cells contacted with the test compound in the absence of WNV or other viruses including *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, do not.

A test compound is provided, preferably in solution. Serial dilutions of test compounds may be used in a series of assays. Test compounds may be added at concentrations from 0.01μM to 1M. A preferred range of final concentrations of a test compound is from 10μM to 100μM.

WNV or other viruses including *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, may be added into the assay by a variety of means. In some embodiments of the invention, it is combined with cells as a protein. The WNV or other viruses including *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, may be added directly to cell culture medium. WNV or other viruses including *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, may be produced from widely available starting materials using well known techniques, such as those described above. A preferred concentration range of the WNV or other viruses including *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, used is about 1μg/ml to 1 mg/ml.

In other embodiments of the invention, WNV or other viruses including *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, is expressed from a nucleic acid, in the cells in the assay. In an non-limiting example, WNV or other viruses including *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, may be expressed within the cells of the assay from a nucleic acid, under the control of an inducible promoter.

The observation of apoptosis in the cells is carried out by methods that detect the hallmark cellular changes or "markers of apoptosis." For example, early during apoptosis, alterations to the cellular membrane result in an externalization of phosphatidylserine (PS) in the cell membrane prior to eventual cell death. The constant exposure of PS during apoptosis makes it a useful "marker of apoptosis," and an attractive target for a variety of detection techniques. Annexin V, which is an endogenous human protein having a high affinity for PS, presents a convenient reagent for identifying cells undergoing apoptosis. Fluorescence-labeled annexin V can be used for histologic and cell-sorting studies to identify apoptotic cells. For example, annexin V can be conjugated to phycoerythrin (PE), a large molecule containing 25 fluors, and one of the brightest dyes used today. PE can be purchased commercially, or isolated from algae by known isolation techniques. Conjugation techniques are known to those skilled in that art, and conjugation kits can be purchased from various vendors, including ProZyme, Inc. (San Leandro, CA). For further details and protocols on conjugating fluorescent proteins for use in flow cytometry and other applications, see Hardy, R., Purification and coupling of fluorescent proteins for use in flow cytometry, *in* Handbook of Experimental Immunology, 4th ed., Weir, Herzenberg, & Herzenberg, eds., Blackwell Scientific Pubs., Boston, 1986, which is incorporated herein by reference. Additionally, radiolabeled annexin V is useful for radiopharmaceutical imaging of apoptosing cells within tumors in the body.

Another "marker of apoptosis" is represented by the free 3'-hydroxy DNA termini, generated by the internucleosomal fragmentation of the cellular DNA by selectively activated DNases. Such free 3'-hydroxy DNA termini are not present in the intact genomic DNA of healthy cells, nor are they present when cells die via necrosis. Apoptosis-associated free 3'-hydroxy DNA termini can be detected *in situ* by the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) assay. For a review of techniques for detecting DNA cleavage during apoptosis, *see* Kaufmann *et al.*, 2000, Methods Enzymol., 322:3 -15, which is incorporated herein by reference.

The internucleosomal fragmentation associated with apoptosis can also be detected by a sandwich assay that uses a pair of monoclonal antibodies specific for two nucleosomal epitopes to capture and detect cytoplasmic nucleosomes onto an enzyme-linked immunosorbent assay (ELISA) plate. Salgame, *et al.*, 1997, Nucleic Acids Res., 25:680-681, which is incorporated herein by reference. This assay is particularly amenable to large scale screening of tissue culture cells.

The apoptosis detection assay may be performed using many different types of cells and delivery of *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, through a variety of means. One having ordinary skill in the art, following the teachings of the Specification, can readily appreciate the several ways to practice this aspect of the present invention. In preferred embodiments of the invention, the assay is performed using tumor-derived cell lines, such as the adenocarcinoma-derived HeLa cell line and the rhabdomyosarcoma-derived RD cell line, or using transformed cells, such as the adenovirus DNA-transformed kidney cell line 293.

A further aspect of the present invention relates to kits for practicing the above described method of identifying compounds which inhibit WNV or other viruses including *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, apoptosis-inducing activity. Kits according to this aspect of the invention comprise a container comprising WNV or other viruses including *Flavivirus* or *Pestivirus* capsid protein, or functional fragment thereof, and at least one of the following: instructions, controls, and photos or figures depicting data. Additionally, a kit may comprise a second container comprising a reagent for detecting apoptosis, such as phycoerythrin (PE)-conjugated annexin V. Alternately, the instructions can direct the user of the kit to utilize any of the many known methods of detecting markers of apoptosis. The kit may also provide the user with the cells to carry out the assay. For example, a vial of cryopreserved tumor cells may be included with the kit.

Diagnostics

There is a great need to develop diagnostic tests by which to detect the presence of antibodies to proteins from WNV or other viruses including *Flavivirus* or *Pestivirus*.

The present invention relates to a diagnostic test in which the presence and/or amount of capsid protein from WNV or other viruses including *Flavivirus* or *Pestivirus* in a test sample is determined. The present invention provides anti-capsid protein antibodies that recognize capsid protein from WNV or other viruses including *Flavivirus* or *Pestivirus*. The presence of capsid protein in a test sample from an individual may also be an excellent indicator of infection.

The present invention relates to methods of identifying individuals exposed to WNV or other viruses including *Flavivirus* or *Pestivirus* by detecting presence of capsid protein in a sample. The antibodies are preferably monoclonal antibodies. The antibodies are preferably raised against capsid protein made in human cells, CHO cells, insect cells or yeast cells.